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Published in:
Traffic

DOI:
10.1111/tra.12601

Publication date:
2018

Document Version
Peer reviewed version

Evolution of protein trafficking in kinetoplastid parasites: complexity and pathogenesis

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Running head: Evolution of kinetoplastid trafficking

Key words: Endomembrane system, evolution, trypanosomes, kinetoplastida, parasitism, plasma membrane, endocytosis, pathogenesis, drug mode of action.

Synopsis

The major differentiation between prokaryotic and eukaryotic cells is a sophisticated system of subcellular organelles in the latter. The vast majority are the product of endogenous evolutionary events, and several paralogous families (Rabs, SNAREs and others) with specific localisations to one or more compartments has allowed predictions of intracellular structure based on gene complements and comparative genomics. Here we consider one lineage, the kinetoplastids, that have been rather well studied, to extract how evolutionary events can mould trafficking.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/tra.12601

Abstract

The kinetoplastida and their close relatives are unicellular organisms prevalent within the eukaryotic biosphere and important for significant impacts on global health, economy and ecosystems. They are, under most models, an early branching lineage. Individual species adapted to highly diverse environments by adopting complex life styles; parasitic species can infect a wide range of eukaryotic hosts, while many relatives are free-living and some autotrophic from acquiring a plastid for photosynthesis. Adaptation is especially evident in the evolution of kinetoplastid cell surface architecture and is supported by endomembrane trafficking and serves as a platform for interaction with environment. Here we summarize and discuss recent genomic and experimental studies of the protein trafficking system in kinetoplastids, with focus on the composition and function of the surface as well as mechanisms for constructing, maintaining and regulating the cell surface proteome. We hope this provides a broad view of how protein trafficking contributes to the intricate and dynamic host-parasite interfaces that are critical for successful environmental adaptation of this highly important lineage.

Introduction

The Kinetoplastida are among a group of highly adaptive organisms, with an extraordinary wide spectrum of habitats ranging from fresh water to parasites of multi-cellular and unicellular hosts (Table 1, Figure 1A). This astonishing capacity for adaptation is greatly reflected in the complex lifestyles developedby individual species, which exhibit an array of morphological changes during life cycle transitions. Along with metabolic adaption, changes in cell architecture play critical roles in establishing such rich biological diversity. Here we consider insights from recent genome annotations and molecular cell biology to propose an atlas for understanding the evolution of trafficking and surface molecules of kinetoplastids in the context of adaption to their individual unique niches.

Taxonomy, parasitism and lifestyles

Kinetoplastids are members of the Euglenazoa, within the Excavata supergroup. With numerous lineage-specific features (Figure 1A), they are among the most divergent taxa from their animal and plant hosts [1]. Significantly, most known kinetoplastids are parasitic, and it is likely that parasitism evolved within Euglenozoa more than once as a result of adaptation [2,3]. The most familiar examples are the trypanosomes and *Leishmania* in the order Trypanosomatida. As causative agents of high-profile human diseases, considerable effort has been dedicated towards management, treatment and understanding of fundamental biology [4].

Most of the studied parasitic kinetoplastids dwell in two hosts through their life cycles. This complex digenous life style requires significant alterations to cellular functions to facilitate host transition and environmental adaptation. Embedded within the Trypanosomatida, and closely related to the Leishmanias, are *Crithidia* and *Phytomonas* infecting insects and plants respectively [5,6]. Free-living bodonids are less well-studied but nearly ubiquitously present in aquatic environments and soil, and form the dominant kinetoplastids in the global oceans [7]. Their ecological significance and full impact to agriculture is almost certainly currently underestimated.

Each trypanosomatid lineage has a unique cell surface. It is of considerable importance to obtain molecular details of the specific

architectural compositions and functional contributions for these distinct surfaces as they are involved in host cell recognition, cell invasion and evasion of host defense systems in mammalian, arthropod and plant hosts. The endomembrane trafficking system builds the surface architecture and supports the complicated and dynamic functions ranging from macromolecule secretion, uptake of nutrients and other materials from the environment, to detection and transduction of external stimuli. Therefore, a systematic understanding of how the endomembrane system in trypanosomatids differentiates between lineages has the threefold potential of providing unique insights into universal functionality, pathogenic mechanisms and evolutionary change.

African and American trypanosomes (*T. brucei* and *T. cruzi* respectively) have surfaces dominated by glycosylphosphatidylinositol or GPI-anchored proteins, a common feature amongst kinetoplastids. Remaining extracellular throughout their entire lifecycle, *T. brucei* and close relatives evolved a highly sophisticated system involving antigenic variation and antibody clearance *via* high flux endocytosis [8,9] to facilitate survival in the host. In the mammalian stage, the surface of *T. brucei* is dominated by the variant surface glycoprotein (VSG) and is also decorated by a number of lower abundance *trans*-membrane and lineage-specific GPI-anchored proteins, several of which are likely receptors for immune effectors [10,11]. In the insect stage, *T. brucei* remodels its entire surface, characterized by the replacement of VSGs by procyclins that are important for persistence within the insect vector[12]. In contrast, *T. cruzi* invades the cells of its host, and its own surface is dominated by mucin-like molecules that defend the parasite from immune and proteolytic attack and are persistent through the life cycle [13]. There are also multiple additional proteins present within the *T. cruzi* mucin coat performing adhesion functions during invasion of host cells.

Leishmania and the *Phytomonads* share the capacity to express a dominant surface glycoconjugate, the lipophosphoglycan (LPG) [14]. LPG is restricted to the insect stages of the life cycle and appears critical in insect transmission and potentially initial entry into the mammalian host. A similar case of functionality is also found in *Crithidia* for an LPG-related macromolecule [15]. Therefore, the GPI-anchor structure, conserved in LPG, VSG, procyclins and mucins, provides a common mechanism for presenting the macromolecules that are crucial for host interactions. *Leishmania* is also capable of secretion of highly glycosylated proteins, facilitating transmission by the sandfly vector. Beyond the trypanosomatida, the free-living *Bodo saltans* and *Euglena gracilis* both possess elements of the LPG biosynthetic pathway within their genomes [16,17]. It is plausible that the parasitic species evolved from free-living forms to initially infect arthropods and only later acquired the ability to infect mammals or vascular plants, and that LPG has a rather fundamental role that is unconnected to parasitism per se, perhaps as a general protective molecule.

The majority of these organisms undergo developmental changes critical for survival, maximal fitness or manipulation of host responses. One fundamental aspect focuses on specific cohorts of surface macromolecules that are predominantly lipid anchored proteins of high abundance and immense diversity. Therefore, individual organisms evolve highly efficient systems for protein trafficking, modification and homeostasis, which likely differentiate functionally in each

organism to match the unique demands for each host. Are such modifications observable in the genomes and/or cell biology of these organisms?

An endomembrane system sculpted by reduction

Over one hundred Kinetoplastida genomes have been sequenced, providing an excellent opportunity to reconstruct transport pathways and predict likely courses of evolution of membrane transport. Sampling is significantly less dense beyond the Trypanosomatida, but is rapidly improving, allowing for more robust predictions.

Many of the major groups of trafficking proteins are paralogous, including Rab and ARF GTPases, SNAREs, tethers, coats and adaptins [18]. In general, functions are well conserved between orthologs across eukaryotes, for example Rab7 is almost always functionally associated with late endocytic/pre-lysosomal transport [19]. This enables the prediction of transport routes with an acceptable and useful level of accuracy, *albeit* precise functions and interactions for specific proteins are somewhat harder to ascertain. Another general tendency of trafficking systems is that paralog numbers scale with genome size [20]. This simple correlation holds for many kinetoplastids and related euglenozoids (Figure 1B), but with notable exceptions such as *E. gracilis* (belonging to class Euglenoidea) in which a massive genome expansion is likely responsible for the anomaly [20]. There is a remarkable similarity in the configuration of the endomembrane system between the last kinetoplastid common ancestor (LKCA) and *E. gracilis* based on comparisons of their Rab and SNARE paralogs. For several Rabs such as Rab1, Rab5, Rab7 and Rab11 in Euglena, multiple paralogs are present suggesting a highly complex endocytic sorting, recycling, ER exit and vacuolar system, consistent with its free-living and flexible lifestyle involving both autotrophy and heterotrophy. However, the Rab cohort of kinetoplastids is reduced compared to the last eukaryotic common ancestor (LECA): orthologs for Rab 8, 20, 22, 24, 50 and RabTitan are absent from the LKCA (Figure 2), despite being present in LECA [21]. By contrast the SNARE cohort is well retained, with Use1 and Syn17 as the only paralogs lost in the LKCA.

At the opposite end of this spectrum lies Perkinsela, which belongs to the group Prokinetoplastida (Figure 1). This curious organism is an intracellular inhabitant of Neoparamaeoba, a parasite of fish. Ultrastructural images suggest a reduced cytoplasmic volume and paucity of distinguishable endomembrane compartments, raising the possibility that Perkinsela lacks much of the canonical organellar apparatus [22]. Another remarkable feature is a highly reduced nuclear genome of less than 10Mb, which encodes only three recognisable Rab proteins, the smallest reported to date. These Rabs resemble Rab1 and Rab2 and hence are likely involved in early exocytosis, begging the question of whether endocytic and intra-Golgi transports occur in this organism, and if so how they are achieved.

Between these two extremes lie the Bodonids, with more conventional genomes and predicted transport pathways. *B. saltans* possesses 35 SNARE- and 34 Rab/Rab-related genes. There are considerable expansions of SNARE Syp7 (6 copies), Rab7 (3 copies) and Rab32-like (5 copies) genes, in addition to a cohort of lineage-specific Rabs. Together these genomic compositions give rise to a highly expanded endosomal/digestive apparatus and also suggest autophagic

pathways, all of which reflect needs to accommodate a variable food supply and survive periods of austerity in a free-living organism (Figure 3).

As trypanosomes gained the parasitic lifestyle, rather than an abrupt collapse of pathways, there is stepwise evolution, with gradual loses and very few gains. The vast majority of changes are simple paralog expansions suggesting no radical rise of novel transport pathways in the lineage, at least since the LKCA. Specially, loss of Rab24 and a tendency to lose both Rab32 and Rab32-like proteins differentiate Typanosomatida from the Bodonids. This feature indicates a decreased flexibility in survival during lean times, likely as a result of adaption to a constant environment provided by the host. A recent addition to the sequenced kinetoplastids is *Paratrypanosoma confusum*, a parasite of mosquitos that diverged prior to the evolution of digenous forms [23]. This organism fits well within the general trend, and possesses 26 Rabs, two copies each of Rab5, 11, 21 and 32 (MCF, unpublished analysis), in concordance with the close relationship to *Leishmania.* Significantly *P. confusum* also cojtains ten Rab proteins that are sufficiently divergent to suggest novel functions.

Continued but distinct losses accompany the emergence of the African trypanosome and *Leishmania/Phytomonas* clades: paralogs of post-Golgi trafficking Rabs such as Rab11, Rab21, Rab32 and SNAREs featured by SynPM, Syx6, Npsn, VAMP7, suggest simplified endocytic and recycling pathways. A similar case occurred in the parasitic Bodonid *Trypanoplasma borreli* where Syx6, VAMP7, Rab32-like genes are lost. Interestingly, the contrary occurs in the American trypanosome, *T. cruzi, where a*dditional Rab11 and VAMP7 paralogs are potentially associated with the contractile vacuole [24]. Overall, these data support the notion that there are gradual evolutionary changes in parasitic species, represented by Bodonida, Leishmania and Trypanosomatida, in which flexibility in energy acquisition and possible austerity pathways are diminished [20].

Exocytosis: simplicity with variation

The fundamental configuration of the kinetoplastid secretory pathway is canonical, with coatomer complexes I and II, tethers and the basic exocytic Rab and SNARE complements all present. However, all trypanosomatids are incapable of synthesizing Dol-P-Glc, the essential donor for the terminal tri-a-glucosyl cap of the lipid-linked oligomannosyl N-glycan precursors [25]. Therefore, the quality control (QC) system within the ER (endoplasmic reticulum) is reduced in comparison to other eukaryotes. Moreover, the oligosaccharyltransferase complex is simplified to a single subunit configuration in trypanosomes, but evolved as two functional paralogs in *T. brucei*, indicating a specification towards the residues flanking the asparagine critical for the N-glycan attachment [26]. Furthermore, as *cis*-splicing is absent in trypanosomes, these organisms lack a conventional ERAD (ER-associated protein degradation) signaling system based on differential splicing and activation of the protein kinase RNA-like endoplasmic reticulum kinase (PERK) in the ER. This notion is also supported by the insensitivity of trypanosomes to reagents that disrupt the canonical ERAD pathways [27–30]. However, a cohort of proteins involved in monitoring protein processing and folding act on VSG and ISG proteins, suggesting some noncanonical protein QC system operates in these organisms [27,30,31].

In other eukaryotes, the COP II complex and a group of receptors

termed p24 together facilitate protein exit from the ER, and with potential differentiation between GPI-anchored and *trans*-membrane domain proteins. As the trypanosome surface is dominated by GPI-anchored proteins (such as VSG in *T. brucei*), it is of significant interest to understand if this process of selective ER exit is conserved. The first clue is provided by the presence of eight p24 paralogs in the *T. brucei* genome, suggesting the potential for considerable functional diversity [32]. In addition, there are two trypanosome specific proteins, TbERAP32 and TbERAP18 at the ER, specifically associated with monitoring the copy number of VSG targeted to the surface [33]. Further there is an extensive group of lineage-specific lectin-related proteins from the invariant glycoprotein family, localised in the ER and potentially involved in QC [34]. In yeast, late steps in exocytosis require the exocyst, and octameric complex. Significantly, a novel subunit of exocyst is present in trypanosomes, and is conserved across the entire lineage [35].

Several paralogs of canonical exocytic SNAREs are present, with up to two Qa-Syx1 and four R-VAMP7 as part of the canonical Syx1-SNAP-25-VAMP7/Synaptobrevin complex in metazoa [20]. Several paralogs of Qb-Npsn and Qc-Syp7 are found across kinetoplastids, with multiple copies in *B. saltans* and the American trypanosomes, suggesting potential roles in trafficking to the plasma membrane as described in plants.

Endocytosis: substitution, replacement and refinement

In trypanosomes, surface maintenance and nutrient acquisition are critical for successful immune evasion and survival within the host, which are greatly dependent on the functions of the endocytic apparatus. Interestingly, recent evidence indicates that some species of extracellular trypanosomes (*T. theileri* and *T. grayi*) [36–38] can survive without VSG, suggesting more diverse mechanisms underlying the immune evasion.

VSG is a homodimer with two GPI-anchors and is distributed over the entire surface of the cell. A second GPI-anchored surface protein, the transferrin receptor, is a heterodimer with only one subunit possessing a GPI-anchor and is retained within the flagellar pocket. This has been proposed as a general model for localisation of specific proteins using 'GPI-valance' [39]. Tests of this model, including the production of a dual GPI-anchored transferrin receptor, lend support, but neither a molecular mechanism nor an exhaustive exploration of the surface protein repertoire have been described. Interestingly, the localization of VSG can be significantly altered by changes to the size of the VSG ectodomain alone, which may suggest that membrane anchoring has a lesser role compared with the architecture of the protein itself [40,41].

Trypanosome endocytosis is exclusively dependent on clathrin-mediated mechanisms that are set around ahave conserved functional core but evolved distinctively. Many proteins described in animals and fungi and required for both clathrin-dependent and independent pathways are absent from trypanosomes [42], including AP-5 adaptin, T-SET, caveolin, multiple paralogs of ENTH/ANTH domain proteins, subunits of the ESCRT complex and many more. However, the trypanosomatids also possess several novel clathrin-associated proteins which play significant roles in endocytosis and subcellular targeting, raising an interesting possibility that a more basal system was present in the LECA, upon which different lineages layered cohorts of proteins to facilitate precise tailoring of function [42,43].

Sorting and dynamic protein turnover

The invariant surface glycoproteins (ISGs) have provided most data on surface *trans*-membrane protein trafficking in trypanosomes. Among this extensive family of type I membrane proteins, ISG75 mediates uptake of the classic trypanocide suramin and ISG65 has utility in diagnostics [44,45]. Notably, compared with the ISGs, VSG is turned over rather slowly [46], indicating a selective sorting mechanism is operating. Ubiquitylation is required for both uptake and degradation of ISGs, and catalyzed by a ligase acting close to the site of uptake [47] and is regulated by at least two deubiquitinases, orthologs of Usp7 and Vdu1. This suggests that ISG turnover is controlled by a rapid and sensitive switch-like mechanism.

Late endocytic systems: More replacements

Nearly all eukaryotic cells possess a lysosomal/vacuolar terminal compartment that serves as a site for protein degradation along with other functions. Most kinetoplastids appear to have a single or very small number of lysosomes. However, multiple Rab7, Qa-Syx7 and Qb-Vti-like paralogs, generally associate with late endosomal transport, have been identified in several kinetoplastids, suggesting multiple transport routes to the lysosome.

Curiously, an essential type I *trans*-membrane protein, p67, replaces the mammalian lysosomal LAMP in trypanosomes. p67 is highly glycosylated and is likely transported *via* an AP-1-dependent pathway that is responsible for maturation and progression through the Golgi complex and to the lysosome [48,49]. Protein analog replacement is not unusual in trypanosomes and frequently does not give rise to significant obvious functional alterations [50,51]. Therefore, function is possibly conserved between LAMP and p67. Additional lysosomal proteins include the major facilitator superfamily of transporters and mucolipin, a member of the transient receptor potential cation channel family, and which are fully conserved.

In close proximity to the lysosome, a late endosome/multivesicular body is present, in which the ESCRT complex is responsible for recognition and processing of ubiquitylated proteins *en route* to the lysosome, and is regulated by the Vps4 AAA-ATPase in a conserved manner [52–54]. However, the ESCRT 0 complex is absent and restricted to animals and fungi [52] raising an interesting question as to how ubiquitylated surface molecules, such as ISGs, are recognised, and presumably deubiquitylated prior to turnover.

Deviations: The cytostome and contractile vacuole

In almost all ciliates, phagocytosis takes place at the cytostome, a surface organelle defined by a microtubule-supported funnel or groove. Similar structures also exist in some Euglenoids and Dinoflagellates [55], although these are generally less elaborated. While feeding on bacteria, *B. saltans* wafts prey into a cytostome [56], which is surrounded by flap-like lips to collect current produced by a flagellum. The cytostome is also present in Crithidia and the basal trypanosomatid *P. confusum* [23], but appears to have been lost in most trypanosomatids, where endocytosis is generally restricted to the flagellar pocket. However, *T. cruzi* epimastigotes possess a cytostome/cytopharynx-like organelle which has an elongated stiletto-shaped structure formed by an invagination of the plasma membrane

along with sub-pellicular microtubules [57]. The structure contains a distinct membrane region that is separated from the neighboring flagellar pocket by the preoral ridge and disappears during metacyclogenesis [58].

Another distinct feature in *T. cruzi* is the contractile vacuole (CV) [59] absent in African trypanosomes and most *Leishmania* species, despite being described in some early work on *Leishmania* [60]. The CV is associated with osmoregulation [61], serving as an internal membranous bladder, periodically filling with hypotonic liquid and being discharged by abrupt contraction through a pore in the plasma membrane. Acidocalcisomes, storage organelles for cations that maintain high concentrations of polyphosphates, also contribute to osmoregulation [62]. In fact, these two types of organelles are often associated with each other physically and functionally [63,64], and while maintaining their membrane identities, are both subject to regulation by Rab8 (absent in all kinetoplastids) and the exocyst complex[65]. Additionally, there are other lines of evidence that suggest selective trafficking of proteins between CVs and the plasma membrane [62]; several trafficking and vacuolar fusion proteins were also observed in the *T. cruzi* CV including SNARE VAMP7A, Rab11, Rab2 and Rab1 [66–69].

Interactions of the trafficking system with therapeutics.

It has recently emerged that several frontline drugs interact with the trafficking system of African trypanosomes, either directly or indirectly. Evolutionary divergence provides a key to selective toxicity and hence therapeutic utility. For example, suramin, a drug first introduced against trypanosomiasis in the first quarter of last century, is recognised by ISG75, entering the cell via receptor-mediated endocytosis, crossing the lysosomal membrane through a major facilitator superfamily transporter [45,47]. The presence of ISG75 and the high rate of endocytosis in *T. brucei* accounts for the selective toxicity of suramin.

Similar mechanisms also underly cross-resistance to melarsoprol and pentamidine, two further frontline therapeutics. There are two transporters, P1 and P2, for purine uptake in *T. brucei* where the *de novo* synthesis pathway is absent. Mutations in TbAT1, encoding P2, gives rise to resistance to melarsoprol [70,71]. P2 also contributes to uptake of diamidines including pentamidine, which is primarily dependent on aquaglyceroporin-2 (AQP2) [45,72,73]. Interestingly, in African trypanosomes, AQP2 and AQP3 paralogs have ~90% amino acid identity, indicating recent duplication. These two paralogs are differentially located, with AQP2 to the flagellar pocket and AQP3 to the entire plasma membrane, and the mutations in AQP2 are exclusively associated with pentamidine sensitivity [74]. Furthermore, uptake of pentamidine is at least partially mediated by endocytosis as the affinity of AQP2 to the drug is exceedingly high [75]. This is thus a second example of a specific and recent evolutionary event underpinning selective drug sensitivity.

Exploitation of the unique aspects of the trypanosome surface and trafficking has underpinned much of the classical therapeutic arsenal, and recent applications of nanobodies has the potential to extend this in a rational manner. Nanobodies (Nbs) are derived from heavy chain-only antibodies of camelids (see recent review [76]), and can be utilized for delivery of drugs and toxins with high efficiency and specificity owing to their biochemical and physical features. An immunotoxin consisting of a nanobody coupled to serum trypanolytic factor Apolipoprotein L-1

(ApoL-1) circumvented resistance dependent on the ApoL-I-neutralising serum resistance-associated (SRA) protein in *T. b. rhodesiense* [77], whilst nanobodies coupled to pentamidine not only enhanced the potency of pentamidine but also overcome resistance to pentamidine dependent on aquaglyceroporin-2 (AQP2) [78]. In addition to targeting, the killing mechanisms for nanobodies also include directly blocking the endocytotic pathway [79]. Overall, identification of additional diverse surface antigens could greatly extend the potential of nanobodies as therapeutics.

Conclusions

Among kinetoplastids, members of protein families associated with delivery or removal of material from the cell surface exhibit the greatest levels of divergence, while those mediating the early secretory/biosynthetic pathways appear to be largely conserved. *T. cruzi* is the only parasitic trypanosomatid that has retained both the cytostome and contractile vacuole, as well as possessing the largest cohort of Rab GTPases and SNAREs from the LKCA. It is yet unclear if this retention has a role to play in the wider range of cells that *T. cruzi* is able to infect in their vertebrate hosts, compared to *Leishmania spp* which are restricted to macrophages (see Table 1).

In all eukaryotic cells, intracellular transport pathways support a wide spectrum of essential activities, and modifying these to suit distinct environments and lifestyles is a key aspect of cellular evolution and adaptation. In the kinetoplastids, we have an exceptionally well sampled group of unicellular organisms, with defined lifecycles and differentiation pathways. Considerable progress has been made in understanding the detailed evolution of transport, and its potential for exploitation for therapeutics. However, it is essential that we extend these analyses to additional kinetoplastids. The large diversity of bodonids and their impact on the environment remains unexplored, and *P. confusum* which appears to link the former with the more derived parasitic trypanosomatids, may provide further insights into the evolution of parasitism in this lineage. The advent of CRISPR/Cas9 technology for gene editing [80] heralds the opportunity to rapidly dissect trafficking and its impact to disease. It is exciting that these fascinating organisms are becoming more and more tractable, and will provide unique insights into basic cell biology and pathology in the years to come.

Acknowledgements

Work in the authors' laboratory is supported by the Wellcome Trust (Investigator award 204697/Z/16/Z) and the Medical Research Council (Project grants MR/N010558/1, MR/K008749/1, MR/P009018/1), which we gratefully acknowledge.

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Figure legends

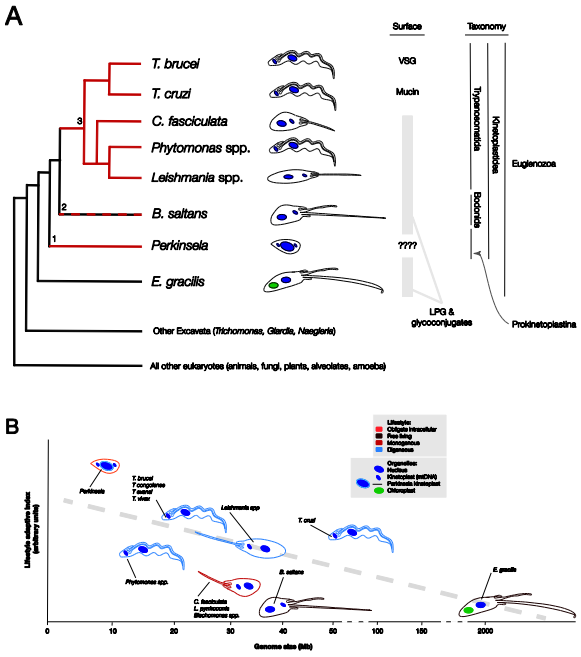
Figure 1: Genome, cellular and lifecycle complexity in trypansomatids. Panel A: Phylogenetic relationships between trypanosomes, Euglena and all other eukaryotes. Red branches indicate parasitic lineages and black non-parasitic. To the right are columns indicating dominant surface molecules, followed by taxonomic groupings. Panel B: Specialisation does not correlate with a reduced genome size. Approximate relative genome sizes are plotted against an adaptation index, which is based on broad concepts of metabolic and environmental flexibility together with specialisation for specific hosts or niches. Organisms scoring higher on this index possess more complex lifecycles, hosts and immune evasion mechanisms (complex surfaces and/or antigenic variation) compared with those lower on the index that are more generalists or, for *Bodo saltans*, free living. Note the breaks in the abscissa as the genome of *Trypanosoma cruzi* is substantially larger than the other entries, and *E. gracilis* which is much larger again. For both panels, organisms in red are monogenous (one host), blue or green digenous (two hosts, animal of plants respectively) and brown free living. Blue ovals are the kinetoplastid (small) and nucleus (large) whilst the light green ovals are chloroplast.

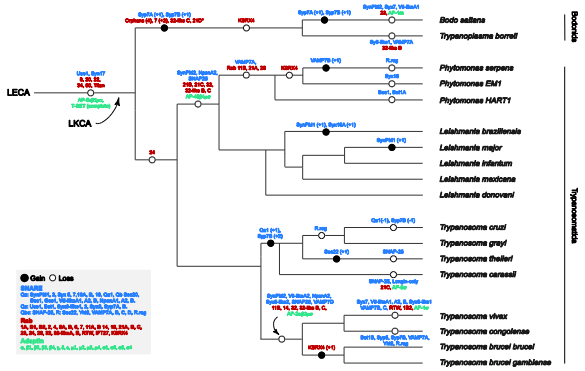
Figure 2: Reconstruction of birth and death of SNARE, Rab and adaptin genes in kinetoplastids. The predicted last kinetoplastida common ancestor repertoires are shown in the box at left. Those subtypes that have undergone an expansion are in **bold**. SNAREs are in blue, Rabs in red and adaptins in teal. Putative points of origin (full circles) and loss (empty circles) are overlaid on a schematic kinetoplastid taxonomy. Numbers in brackets indicate the minimum number of copies of genes that are predicted lost (-n) or gained (+n). The evidence for losses and gains are based on data presented in [20].

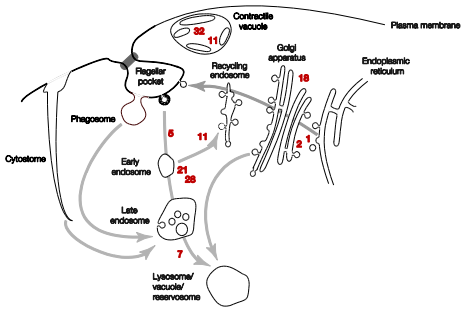
Figure 3: Trafficking pathways of kinetoplastids. The endomembrane system of kinetoplastids is broadly conventional, in the sense that recognisable subcompartments, with essentially conserved functions corresponding to endosomal and exocytic trafficking can be readily recognised. These compartments/functions can also be defined at the molecular level with conserved marker proteins; several Rab GTPase locations are shown in red as an example. Major changes/novelties however are present in *T. cruzi* and include the presence of a cytostome and contractile vacuole (blue). Further, phagocytic activity has been reported

for *Bodo saltans* (brown), *albeit* with no known molecular details.

Table 1: Summary of the diversity of lifestyles of kinetoplastids and relatives. Kinetoplastids are grouped and coloured according to conventional phylogeny. Major cell surface proteins of many newly sequenced kinetoplastids, especially the bodonids and some *cruzi* group members as well as outgroup species and *E. gracilis* are as yet essentially described, and descriptions are based in predictions from the genome sequence.







Organism	Host	Vector	Flagella	Cell surface proteins	Life style
Naegleria gruberi	heterotroph	n/a	Multiple	unknown	Free living
Euglena gracilis	auto+heterotroph	n/a	2 free	unknown	Free living
Perkinsella	Neoamoeba	n.a	0	unknown	Intracellular parasite
B. saltans	heterotroph	n/a	2 free	unknown	Free living
T. borreli	fish	leech	2 free	unknown	extracellular, blood-stream
P. confusum	Mosquito		1 free	unknown	extracellular
P. serpens	plant	hemipteran insects	1 free	gp63	extracellular, phloem
P. EM1	plant, symbiotic	hemipteran insects	1 free	gp63	extracellular, latex tubes
P. HART1	plant	hemipteran insects	1 free	gp63	extracellular, phloem
L. braziliensis	vertebrates	phlebotomine sandfly	1 free	gp63, LPG; amastin	intracellular, macrophag-es
L. major	vertebrates	phlebotomine sandfly	1 free	gp63, LPG; amastin	intracellular, macrophag-es
L. infantum	vertebrates	phlebotomine sandfly	1 free	gp63, LPG; amastin	intracellular, macrophag-es
L. mexicana	vertebrates	phlebotomine sandfly	1 free	gp63, LPG, amastin	intracellular, macrophag-es
L. donovani	vertebrates	phlebotomine sandfly	1 free	gp63, LPG; amastin	intracellular, macrophag-es
T. cruzi	vertebrates	triatomine bugs	1 attached	gp63, mucins, *trans*-sialidases; amastin	intracellular, many cell types
T. grayi	reptiles, mammals	tsetse fly	1 attached	*trans*-sialidases	extracellular, blood-stream

T. theileri	mammals	ticks	1 attached	unknown	extracellular, blood-stream
T. carassii	fish	leech	1 attached	mucins	extracellular, blood-stream
T. vivax	mammals	tsetse fly	1 attached	VSG; Procyclins	extracellular, blood-stream
T. congolense	mammals	tsetse fly	1 attached	VSG; Procyclins	extracellular, blood-stream
T. brucei brucei	mammals	tsetse fly	1 attached	VSG; Procyclins	extracellular, blood-stream
T. brucei gambiense	mammals	tsetse fly	1 attached	VSG; Procyclins	extracellular, blood-stream

Table 1: Summary of the diversity of lifestyles of kinetoplastids and relatives. Kinetoplastids are grouped according to phylogeny as in Figure 1. Major cell surface proteins of many newly sequenced kinetoplastids, especially the bodonids and some cruzi group members as well as outgroup species *N. gruberi* and *E. gracilis* are as yet not described in any detail.